DNA Profiling with PCR

OBJECTIVES

- To review the structure and function of DNA.
- Understand and perform the polymerase chain reaction (PCR)
- To gain experience using the micropipettes, thermocycler, and gel electrophoresis
- To review the principles of Mendelian genetics
- To explore the mechanisms for DNA profiling

THIS IS A COMPLICATED EXPERIMENT. TO SUCCEED, YOU MUST CAREFULLY READ THIS HANDOUT BEFORE COMING TO LAB!

Background:

DNA profiling is a general term encompassing a variety of molecular genetic methods used to distinguish one human being from another. This powerful tool is now routinely used to investigate crime scenes, missing persons, mass disasters, human rights violations, and paternity testing. For example, crime scenes often contain biological evidence, such as blood, semen, hairs, or saliva, from which DNA can be extracted and amplified (copied). If the DNA profile from the evidence matches the DNA profile of a suspect, the individual is included as a suspect; if the DNA profiles do not match, the individual is excluded from the suspect pool.

The human genome consists of ~3 billion bases. And more than 99.5% of this genome does not vary between human beings. Thus the challenge of a DNA profile is to focus on that small percentage of the human DNA sequence (<0.5%) that does vary; these regions are known as polymorphic (“many shapes”) sequences. By convention, the polymorphic DNA sequences used for DNA profiling are neutral and do not control any known traits or have known functions.

The DNA used in profiling are non-coding regions that contain segments of short tandem repeats or STRs. STRs are very short sequences that are repeated a variable number of times. For example, the TH01 region contains a variable number of repeats of the sequence TCAT.

More than 20 different forms or alleles have been identified for the TH01 region, each differing in the number of repeats present in the sequence. Each of us has two of these alleles, one inherited from our mother, and one from our father. The example below shows hypothetical genotypes from two individuals. Individual A, at left, has five repeats in one of their alleles, and three in the other. Individual B, at right, has six repeats in one allele, and ten in the other. Thus examining this region of their DNA would allow us to distinguish these two individuals.

In our lab today we will simulate the process of DNA profiling using a similar region of DNA, known as BXP007, to distinguish multiple DNA samples, including one from a “crime scene”, and samples from four potential suspects. As the DNA left at crime scenes is usually very small amounts of total genomic DNA, we will first need a technique to focus on just the BXP007 region, and make many copies of just this area. We will use a process known as the polymerase chain reaction (PCR) to both focus on just this region of the genome, and to make copies of it.
What is PCR?

In 1983, Kary Mullis at Cetus Corporation developed the molecular biology technique known as the **polymerase chain reaction** (PCR). PCR revolutionized genetic research, allowing scientists to easily amplify short specific regions of DNA for a variety of purposes including gene mapping, cloning, DNA sequencing, forensics, and gene detection.

The objective of any PCR is to produce a large amount of DNA in a test tube starting from only a trace amount. A researcher can take trace amounts of genomic DNA from a drop of blood or a single hair follicle, for example, and make enough to study. Prior to PCR, this would have been impossible! This dramatic amplification is possible because of the structure of DNA, and the way in which cells naturally copy their own DNA.

DNA in our cells exists as a double-stranded molecule. These two strands, or sequences of bases, bind to one another in a very specific, predictable fashion. Specifically, A’s will only pair with T’s, and C’s will only pair with G’s. Thus if you know the sequence of one strand of DNA, you can accurately predict the sequence of the other.

Both DNA replication and PCR take advantage of this predictability. In your cells, one strand of DNA is used as a template to copy the sequence of your DNA from every time a cell divides. PCR does essentially the same process, using one strand of your DNA as a template to produce copies of its sequence.

PCR is conducted in three steps: 1) Denature the template DNA, 2) Allow the primers to anneal, and 3) Extend (copy) the template DNA. In the first step, the template DNA is heated up to break the hydrogen bonds holding the two strands together. This allows each strand to serve as a template for generating copies of the DNA. In the second step, the temperature is reduced to allow the primes to anneal, or bind, at their complimentary sequence on the template. (Primers are short, specific pieces of single-stranded DNA that provide a starting point for the enzyme that will do the ‘copying’. Our primers today bind to DNA on either side of the BXP007 locus.) In the third step, the temperature is raised again to allow the enzyme to bind at the primer and add bases to the growing DNA molecule. These three steps are repeated between 20 and 40 times in a special instrument called a **thermocycler**.

The power of this process is that it results in exponential growth. After the first round of copying, a single DNA molecule will have produced two identical copies. These two copies will generate four molecules in the next round. Those four molecules will create eight, and so on. Thus in 30 cycles we generate literally millions of copies of DNA from each template molecule! We will be able to visualize these millions of copies using a process called DNA electrophoresis. (The process of electrophoresis is discussed in Part 2 of this lab handout.)

**Polymerase Chain Reaction (PCR)**

- **Denature**
- **Anneal**
- **Extension**

What will we learn from this PCR?

By visualizing our results using DNA electrophoresis, we will be able to determine whether or not any of the suspect’s samples matched our “crime scene” at this locus. But it is critical to note that this does NOT mean this suspect committed this crime. There may be thousands, or even millions, of other people with similar sets of alleles at this locus. Thus it becomes very important to consider the **power of discrimination** provided by a particular profiling technique. The power of discrimination is the ability of the profiling to distinguish between different individuals. As an example, one region may be able to tell the difference between one out of 1,000 people, whereas two regions considered together may be able to discriminate between one out of 10,000 people. The larger the number of regions examined, the more powerful the ability to discriminate. For this reason, most DNA profiling currently done in the U.S. utilizes 13 different regions in a system known as CODIS (Combined DNA Index System).
PROCEDURE

Part 1: Preparing your PCR Samples

1. Each group will be provided with 6 tubes on ice, and 5 unlabeled 0.2 ml PCR tubes. You will need to label each of these tiny PCR tubes with your group’s letter, and a number corresponding to the table below.

2. After your instructor has demonstrated their proper use, use a micropipettor to add 20 ul of DNA to each tube as indicated in the table below. Be sure to use a clean tip for each addition to avoid cross-contamination of samples.

3. Again using a clean tip for each addition, add 20 ul of the PCR master mix (tube labeled MM) to each of your PCR tube. Pipette up and down gently to ensure that these solutions are well mixed.

4. Cap your tubes tightly and place them in the thermocycler. When all groups have finished assembling their tubes, your instructor will start the thermocycler.

Table 1: Contents of the PCR Tubes

<table>
<thead>
<tr>
<th>Tube Number</th>
<th>DNA Template</th>
<th>PCR Master Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20 ul Crime Scene DNA</td>
<td>20 ul Master Mix (MM)</td>
</tr>
<tr>
<td>2</td>
<td>20 ul Suspect A DNA</td>
<td>20 ul Master Mix (MM)</td>
</tr>
<tr>
<td>3</td>
<td>20 ul Suspect B DNA</td>
<td>20 ul Master Mix (MM)</td>
</tr>
<tr>
<td>4</td>
<td>20 ul Suspect C DNA</td>
<td>20 ul Master Mix (MM)</td>
</tr>
<tr>
<td>5</td>
<td>20 ul Suspect D DNA</td>
<td>20 ul Master Mix (MM)</td>
</tr>
</tbody>
</table>

Next Steps: The thermocycler will heat and cool your samples 40 times, making millions of copies of any regions of DNA that match the primers. This process will take several hours. When it has finished, your instructor will place the samples in the fridge for you to analyze during your next lab session. In the meantime, answer the questions on the following page and read Part 2 of this protocol carefully before coming to lab next week.
Questions to Answer:

1. You added a PCR “Master Mix” to each sample. What sorts of ingredients must be in this mix to allow the PCR reaction to work? For each ingredient you identify, be sure to specify its function in the reaction.

2. Consider two parents. One parent has 5 repeats at the TH01 locus on both of her homologous chromosomes. The other parent has 10 repeats on both of his.

   a. If you were provided with DNA from each of these individuals, and performed a PCR with primers specific to each end of the TH01 region, how long would the fragment you generated be for each of the parents? (Ignore the length of the primer in your calculations!)

   b. If you were provided with DNA from a child of theirs, what would you expect the results of your PCR analysis to look like? (That is, what size fragments would you generate?) Explain how you are able to make this prediction!

   c. Now consider a crime scene in which two offspring from these same parents (siblings!) are both suspects. Could a DNA profile based on just this TH01 region determine which sibling had committed the crime? Why or why not? If not, how might you improve your profiling to be able to distinguish these two individuals?
DNA Profiling with PCR - Part 2

OBJECTIVES:
- To review the structure and function of DNA
- Understand and perform the polymerase chain reaction (PCR)
- To gain experience using the micropipettes, thermocycler, and gel electrophoresis
- To review the principles of Mendelian genetics
- To explore the mechanisms for DNA profiling

Background: Recall that last week in lab we used the polymerase chain reaction (PCR) to amplify (copy) a specific region of DNA from a “crime scene” and four “suspects”. We targeted a specific region of DNA, the Bxp007 locus, known to contain variable numbers of short tandem repeats (STRs). As this region is highly variable between individuals, it should allow us to determine if any of our “suspects” are a close match to the DNA left at the “crime scene”!

How can I tell if my PCR worked? Unfortunately, you cannot see individual DNA molecules with either your naked eye or our light microscopes. So we will need another technique to allow us to determine whether or not our PCR was successful. The technique we will use is known as DNA electrophoresis. (See diagram at right.)

How does DNA electrophoresis work? In DNA electrophoresis, a collection of DNA molecules is placed in wells at one end of a dense matrix called an agarose gel. An electrical current is then conducted across the gel. As DNA is a negatively charged molecule, it will be drawn towards the positive pole. (Opposites attract!) Thus our DNA will move through the gel, and smaller fragments will travel faster, and therefore farther, than larger fragments. This allows us to sort the DNA fragments from each PCR based on their size.

What will I see as my gel runs? We will mix our PCR products with a colored loading dye. This loading dye will make it easier to see and load our samples. In addition, the dye itself is also negatively charged; thus it will also migrate through our gel when the current is applied. You will see this dye moving. The DNA produced in the PCR reaction, however, is still not visible as it has no natural color. To see these DNA molecules, have added a fluorescent stain to the gel. This stain will bind to the DNA molecules as they pass through the gel, allowing us to see them on a special light when the gel is finished.

Part 1: DNA Electrophoresis
Note: We will work today in the same group of 4-5 students, with each group running their own PCR products on their gel. Make sure everyone has a chance to practice with the micropipettes and load a sample or two!

Part A: Pouring the Gel
1. Obtain an electrophoresis kit from the back counter. Each lab group will need one kit.
2. Remove the tray from the electrophoresis chamber. Use gel casting trays to carefully seal each end of the tray.
3. Place the comb in the tray, being sure it fits tightly in the notches on the edge of the tray.
4. Your instructor will provide melted agarose. **Handle this solution with care as it is hot!** Carefully pour the melted agarose into your tray, filling it up to 2-3 mm past the bottom of the “teeth” on your comb (you will see a fine line on the side of the tray – you should fill to this height).
5. Allow the gel to cool undisturbed on a level surface for ~10 minutes. The gel is ready when it is slightly opaque and solid to the touch.
6. When your gel is cool, gently remove the comb. You can then remove the gel, in its tray, from the casting stand.
7. Transfer the gel, in its tray, to the electrophoresis chamber. Fill both ends of the chamber with buffer until the gel is completely submerged. Your gel is now ready to load!

**Part B: Preparing your Samples.**
1. Carefully retrieve your PCR samples from last week. (Remember that these tubes have thin walls and should be handled gently.)
2. Using a fresh tip each time, carefully add 10 ul of Orange G loading dye (LD) to each sample and mix well. On these pipettes, the dial should read “1-0-0” if your pipette is set correctly. Remember to depress the plunger only to the first stopping point when drawing up liquids.

**Part C: Loading your Gel.** When your group has reached this point, your instructor will demonstrate how to load a gel by loading an allele ladder on your gel. This ladder contains the full set of alleles for this locus and can be used to identify the alleles amplified by your PCR. After your instructor has demonstrated the process, you may proceed.

1. Using a fresh tip each time, carefully remove 20 ul of the PCR/Loading dye mix. (Your pipette will read “2-0-0” this time.) Place the pipette tip at the top of the well, and slowly dispense the solution, allowing it to sink into the bottom of the well. Note that one of the most common errors is to push the tip too far down and poke a hole in the bottom of the gel!
2. Record which well you placed the sample in, and continue as above to load the remaining four samples.

**Part D: Running the Gel.**
1. When your gel is loaded, carefully place the cover on your electrophoresis chamber, taking care to align red with red and black with black.
2. Plug the ends of the wires into a power supply. We will run our gels at 200V for approximately 20-25 minutes. At the end of this time, turn off the power supply.

**Part E: Visualizing the Gel**
1. When your gel has finished running, carefully unplug it and drain the buffer into the beaker provided (it can be reused).
2. One member of your group should put on gloves and carefully transfer the gel to one of the gel illuminators located throughout the room. Lower the cover on the illuminator and turn the light intensity dial to the right to visualize your gel. **Remember that when you wear gloves in the lab you should NOT touch any common items (doorknobs, pens, faucets, etc.) with gloved hands!**
3. Sketch an image of the gel in the space provided, taking care to carefully note any difference in the distance the bands traveled. You sketch should, of course, indicated which sample was in each lane. In addition, you may find it helpful to take a picture of the gel.
4. Dispose of your gel as indicated by your instructor and use a KimWipe to gently clean the surface of the illuminator. Work from your lab notebook to analyze your results and record them in the class data table.

**Clean-up:** Before you leave the lab today, make sure your electrophoresis chambers and gel trays have been rinsed with tap water, dried, and returned to their storage boxes. Place these boxes on the carts in the classroom. Micropipettes and unused pipette tips should also be returned to their boxes. Take care to wipe down your workspace if any solutions have been spilled. The empty plastic tubes may be thrown in the trash.
Questions to Answer:

1. In the table below, sketch the results you see AFTER your gel has been stained. Based on this sketch, fill in the right-hand column, indicating whether or not your PCR worked as expected.

<table>
<thead>
<tr>
<th>Sketch of gel</th>
<th>Lane #</th>
<th>Sample</th>
<th>Results?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>Allele Ladder</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Crime Scene PCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Suspect A PCR</td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>Suspect B PCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Suspect C PCR</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Suspect D PCR</td>
<td></td>
</tr>
</tbody>
</table>

2) Did all of your PCR’s work? If not, what might have gone wrong in your reactions?

3) Based on your results, is it possible to connect any of the suspects to the crime scene? Explain. Can you exclude any suspects?